inhibitors, and crystallization promoting conditions such as elevated temperatures will tend to drive the conversion to a more crystalline product.

Solid PCA calcium phosphate devices.

In another application of the invention, solid PCA calcium phosphate compositions are prepared, either *in vivo* or *ex vivo*. The first method of making a solid is to compress the unreacted precursors of the PCA material. ACP converts to PCA calcium phosphate once the pellet has been exposed to an aqueous environment (e.g. *in vivo* implantation). The second method of production involves compressing already converted PCA granules into a desired shape. The material can also be formed by any other pellet fabrication method known in the pharmaceutical industry. Once the shape has been fabricated, it can be modified in the following ways: A coating can be added to the shaped material. Therapeutic substances are absorbed to the solid material. There can also be further modification of the shape and texture of the pellet. Sterile pellets may be prepared through the use of presterile components or by terminally sterilizing the pellet. All variations to the solid PCA calcium phosphate are considered within the scope of the present invention.

Methods of Pellet Production. In one embodiment, compressing the unreacted precursors of the PCA material produces the prehardened pellet. The first component is an amorphous calcium phosphate. The second component is the promoter. The preferred promoter is dicalcium phosphate dihydrate (DCPD). In other cases, the promoter may be other calcium phosphates such as crystalline HA. The two components are compressed and molded into the desired shape by any suitable method. Preferred embodiments of compression and molding include hand-held presses and hydraulic presses as described in examples 32 and 33. The pressure of the compression is dependent on what characteristics are desirable for the pellet. For instance, lower pressures are favorable for a pellet that is quickly resorbable. Other methods of pellet fabrication known in the pharmaceutical industry are also acceptable. The compressed object of desired shape most preferably reacts endothermically at 37°C in vivo to form PCA calcium phosphate. A conversion of ACP in the presence of a promoter occurs under these conditions during this

reaction to form PCA calcium phosphate.

In another embodiment, PCA calcium phosphate is formed *in vitro*. An amorphous calcium phosphate in the presence of a promoter and a limited volume of aqueous medium is converted to poorly crystalline apatitic calcium phosphate. In the most preferred embodiment, the PCA material is hardened at 37 °C. Once the PCA material is a solid, it is lyopholized. The dry material is then ground for a specified amount of time in a grinding chamber. Other methods of grinding, such as a mortar and pestle, are also acceptable. The powder is then formed into a pellet or other desired shape by the methods described above.

PCA material may also be prepared by combining an amorphous calcium phosphate with a promoter and a biologically suitable aqueous medium. At this time, the PCA material, as a paste or putty consistency, is molded by any suitable method into the desired form. Once the material is molded, it is then hardened most preferably at 37°C. A range of temperatures below and above 37°C is also acceptable. Once the molded object is a solid, it is then lyopholized. The object is lyopholized because the presence of water in the pellet may cause the material to be more unstable and have a tendency to become more crystalline.

Once the PCA material is produced, it is formed and hardened, and then lyopholized as described previously. In some instances, it may be unstable and tend to become more crystalline and eventually converts to hydroxyapatite. The prepared solid PCA calcium phosphate can then be stored either wet or dry. Stability issues surrounding the storage of PCA material include temperature, lyopholization, the use of inhibitors, and whether the material is wet or dry. Lyophilization improves the stability of the PCA material because the presence of water is cause for the conversion reaction. Lower temperatures will enable the PCA material to be more stable when compared to the stability at room temperature or in vivo. Ideal conditions include dry

storage of pellets at room temperature with no exposure to moisture. The PCA material can also be stored in an aqueous medium for up to 30 days, at room temperature, and pH = 7. FTIR and XRD analysis may be conducted on the PCA material to monitor the stability of the PCA material during the storage period. The presence of peaks at 563 cm-1, 1034 cm-1, 1638 cm-1, and 3432 cm-1 (FTIR) should remain unchanged.

Medical Uses of Pellets. The solid PCA calcium phosphate material can be used in many different applications, depending on the details of the situation. The first application applies to orthopedic implants. Pellets, plates, screws, granules, bone void fillers and other forms are appropriate for orthopedic applications. The pellets, plates, and screws can be of various shapes and sizes.

Bone void fillers are gently packed into voids in the bone, which are surgically created defects or defects created from traumatic injury, tumors or other diseases. Sand grain granules (1-2mm) of PCA calcium phosphate can also be used in additional hard tissue sites. The granules are particularly useful in alveolar ridge repair and hairline fractures. However, other applications include, but are not limited to tibial fractures, maxillo and cranial indications, extraction socket voids, and later spinal fusion. A significant advantage to granules is that they can be arranged to fit into small areas where bone regeneration is needed. Also, the sand size granules are used to anchor prosthetics since they can shift and settle into the areas where implanted and serve to hold the various medical devices in their proper locations. In addition, the pellets can be mixed with PCA paste for implantation purposes. The use of these solid resorbable implants also eliminates the need for metal implants in the body.

A second application for solid PCA calcium phosphate is to provide support matrices for living tissues. These matrices can be used to promote cell growth, cell transplant and cell therapy. By supplying the appropriate cells onto the support matrix of prehardened PCA material, the cells are effectively delivered to the desired implant site. Cells may be seeded into the PCA in vitro or in vivo depending on what is appropriate for the given indication. The use of living cells in the body promotes self-healing through tissue regeneration.

are representative XRD from reactions 1-2 and 1-4. The use of two different grain size hydroxyapatites as participating promoters yielded similar results as with different grain size DCPDs (see Example 10) That is, the larger grain size hydroxyapatite hardened more slowly and less completely than the smaller grain size hydroxyapatite.

Example 2. This example demonstrates the use of a neutral apatitic calcium phosphate as a promoter for the conversion of ACP to the inventive PCA calcium phosphate to promote bone growth *in vivo*. Stoichiometric hydroxyapatite is mixed with reactive ACP as described in Example 1-4. Hydrated precursor paste is applied to animal subjects as described in Examples 15, 16 or 19. Bone healing and biocompatibility is monitored as described at the time points indicated.

Example 3. This example demonstrates the production of PCA calcium phosphate from ACP using a number of different passive promoters.

Highly reactive ACP was prepared according to Example 5. ACP was mixed with the specific promoter at a ratio (wt/wt) of about 5:1 or 1:1 (see Table 2) for 5 minutes in a SPEX laboratory mill. Water (0.75 - 0.85 mL) was added and mixed to form a putty. The mixture was then formed into a ball, wrapped in moist tissue paper and heated to 37° C for at least 30 minutes. After 30 minutes and at various time points thereafter the paste was monitored for hardness. Figure 13 is a representative XRD from sample 2-4 employing an alumina promoter. In this figure the alumina peaks can be seen superimposed over the standard PCA calcium phosphate profile.

shown in Figure 4a. The Ca/P ratio measurement of this material after the heat treatment was determined to be 1.575, using a method of quantitative electron microprobe analysis. The overall morphological and ultrastructural properties of the amorphous material was confirmed by transmission electron microscopy as shown in Figure 1. Note the "amorphous" appearance of the material with absence of sharp edges separating each granules with certain portion of the material to exhibit shapeless form (arrows).

Example 6. ACP was synthesized as described in Example 5 above, with the exception that solutions A and B were prepared in the following way: Solution A was prepared at room temperature by the rapid dissolution of 90.68 g of $Ca(NO_3)_2 \cdot 4\ H_2O$ in 1.2 liter of carbonated distilled H_2O. Solution B was prepared by dissolving 40.57 g of K_2HPO_4 in 1.53 liters of distilled H_2O, containing 24 ml of 45 vol. % KOH solution. Chemical and physical properties of the product amorphous calcium phosphate resulting from this procedure were similar to those of the material prepared accordingly for Example 5.

Example 7. ACP was synthesized as described in Example 5 above, with the exception that solutions A and B were prepared in the following way: Solution A was prepared at room temperature by the rapid dissolution of 10.58 g of $Ca(NO_3)_2.6\ H_2O$ in 0.15 liters of carbonated distilled H_2O at pH greater than 9.0, as adjusted by NaOH. Solution B was prepared by dissolving 7.8 g of $(NH_4)_2HPO_4$ in 0.35 liters of distilled H_2O.

Example 8. This example describes the preparation of PCA calcium phosphate of the invention with manual mixing of the dry reactants.

Dicalcium phosphate dihydrate (DCPD) was prepared at room temperature by the rapid addition of solution B (17.1 g $Ca(NO_3)_2 \cdot 4\ H_2O$ (calcium nitrate tetrahydrate) in 250 mL distilled water) to solution A (10 g $H_9N_2O_4P$ (diammonium hydrogen phosphate) in 500 mL distilled water at a pH of 4.6-4.8) with constant stirring. Immediately thereafter, the sample was filtered using filter paper (0.05 sq. m) with medium filter speed and a vacuum pressure of about 10-2 torr. The material formed a thin cake which was washed with about

phosphate calcium phosphate.

PCA calcium phosphate calcium phosphate was prepared according to Example 9 to form a paste. The paste was placed into a 6 (dia.) x 10 (depth) mm hollow Teflon® tube submersed in 37 °C water for 30 minutes. The hardened PCA calcium phosphate was then removed from the tube and placed in water at 37 °C for 1 hour and then, while still moist, placed vertically on an Instron 4206 having a dual 10 kg/15 ton load cell. Compressibility was determined using a crush test. Approximately, 200-250 N were required to bring the sample to failure. This force corresponds to a compressive strength of 7 - 9 Mpa.

Poly(lactide) whiskers are prepared with average dimensions of about 5-100 μm diameter by 10-250 μm length. The whiskers are mixes with poorly crystalline hydroxyapatite paste prepared as described above at a concentration 10% wt/wt. The composite paste is hardened overnight at 37 °C in moist environment. When tested for compressibility, the material is found to have improved compressibility over the non-composite PCA calcium phosphate.

Example 14. These examples demonstrate the effect of fluid volume on the consistency and reactivity of injectable paste to be used in the formation of bone substitute material. Each of the pastes were prepared as described in Example 8, above, and the consistency and rate of reaction at room temperature and 37 °C were determined. Observations are reported in Table 4.

Table 4.
Formability, Injectability and Reactivity of Hydrate Precursor.

Example No.	water volume (mL)	formability	injectability	hardening time at various temps. (4° C/RT/37° C)
14-1	0.7	- crumbles	-	-/-/-
14-2	0.8*	+++ easily formed paste	+	>60 min/>60 min/30 min
14-3	0.9*	++ toothpaste	++	>60 min/>60 min /30 min
14-4	1.0	+ liquid toothpaste	+++	>60 min/ >60 min/30 min

* Under some circumstances (e.g., evaporation) these samples may dry out somewhat over a period of one hour at room temperature. In such cases, additional water may be added to restore the original consistency.

Example 15. Implantation and Resorption of PCA calcium phosphate in a Subcutaneous Site. This example demonstrates the resorption of the inventive

an Intramuscular Site. This example describes the preparation of PCA calcium phosphates that have varied *in vivo* resorption times as a result of varied grinding times.

Individual dry precursors, ACP and DCPD were prepared as described in Example 8. Several different formulations of DCPD and ACP were then prepared by i) grinding DCPD for 15 sec, 30 sec, 1 min, 2.5 min, or 5 min in a SPEX grinder; ii) combining the ground DCPD 1:1 with ACP; and iii) grinding the mixture for an additional 15 sec, 30 sec, 1 min, 2.5 min, or 5 min, respectively. Total grinding times for the different preparations were therefore 30 sec, 1 min, 2 min ("Type 2" powders), 5 min, and 10 min ("Type 10" powders).

The PCA calcium phosphate, sterilized in powder form by approximately 2.5 Mrad of gamma irradiation, was prepared by taking the material in powder form and mixing with sterile water or saline and forming it into approximately 1 cm disks 2 mm thick and incubated for a minimum of 30 minutes at 37 °C. Disks were implanted into adult male New Zealand White Rabbits immediately following fabrication.

Animals were assigned to dose groups which contained 3 males for a total of 15 animals. The implants were assigned to the rabbits randomly. 10-15 minutes prior to the surgery, the animal was premedicated with xylazine (10 mg/kg, i.m.). The animal was then given ketamine (50 mg/kg, i.m.). The dorsal surface of the animal was clipped free of hair and washed with a betadine surgical solution and alcohol. Before the surgery the animal was monitored to be sure that is was properly anesthetized. To do this, pressure was applied to the foot pad. When there was no response, the animal was properly anesthetized. Throughout the procedure, the animal was monitored for whisker twitching and the toe-pinch reflect, which indicated that the animal was not waking up.

Using aseptic technique and a scalpel blade, an incision 1-2 cm in length was made in the skin over the m. longissimus lumborum (which lies along both sides of the spine). When the incision was made, the underlying fascia and muscle was also cut to allow the sample to pas into the muscle. The sample disk was placed directly into the muscle, being sure that the entire implant was

26°, 28.5°, 32.0° and 33.0°. It is interesting to note that there is no change in the spectra after 75 minutes of reaction, indicating that the reaction essentially complete in little more than one hour. The X-ray diffraction pattern of the bone substitute material of the invention (Fig. 5d) can be compared to that of naturally occurring bone, shown in Fig. 7. The two spectra are nearly identical.

Example 19. Implantation and Resorption of PCA calcium phosphate in a Bony Site

The purpose of this study was to assay resorption and ossification of PCA calcium phosphate in a bony implant site. The method is also useful for testing the resorption and ossification properties of PCA calcium phosphate formulations and composites of the invention.

The test article used was a PCA calcium phosphate formulation prepared as described in Example 8. The ACP and DCPD were mixed in the specified proportions and ground for 1 minute, 30 seconds in the SPEX grinder equipment.

Adult (> 5 month old) NZW male rabbits were held in quarantine and acclimatized for a minimum of 10 days prior to the initiation of the study. Animals were individually housed in suspended stainless steel cages. Wood shavings were used in dropping pans under the cages. Prior to initiation of the study, animals were assigned to groups or treatments randomly and were identified by a numbered ear tattoo and by a corresponding cage card. All animals had single defects placed in one tibia. Timepoints for evaluations were 2, 4, and 8 weeks (2 animals at each timepoint). Surgery was performed under full anesthesia and aseptic surgical conditions.

After obtaining adequate anesthesia (e.g., ketamine/xylazine to effect), using aseptic technique, an incision was made over the lateral proximal tibia. The soft tissue was deflected away and the bone exposed. Using an approximately 5 mm trephine in a low speed dental handpiece with irrigation (0.9% physiologic saline) as needed, a ~5.5 mm diameter hole was cut through the cortical portion of the bone. The bony disk was dissected free from the cortex and the site was prepared for implantation. The hydrated precursor material in paste form was placed into the defect. Defects in control animals

Example 24. Different Hydrating Agents Effects on Hardening and Final Product.

A hydrated precursor (ACP and DCPD) was prepared as described in Examples 8, 9, or 10, with the exception that a variety of hydration media were used. Samples were then tested for hardness and completeness of reaction at various time points. In all cases, 1 g of the mixed precursors were hydrated with 0.75 - 1.0 mL of hydration medium to produce a paste. Table 9 summarizes the results and demonstrates that a variety of aqueous based liquids, and in particularly physiologically acceptable media, may be used in the preparation of PCA calcium phosphate.

Table 9
Effect of Hydrating Agents

Hydration Medium	Incubation Time	Hardening
Tris	30 min	hard
0.9M NaCl	30 min	hard
MEM	30 min	hard
MOPS	30 min	hard
HEPES	30 min	hard
BUFFERALL	30 min	hard
PBS	30 min	hard

Example 25. ACP was prepared as described in Example 5, with the exception that the heating the ACP to 450° C was carried out for either 1 hour or 6 hours. Following heating the ACP was prepared for reaction with DCPD as described in Example 8. Hydrated PCA calcium phosphate precursor prepared with ACP heated for 6 hours was found not to harden after 2 hrs at 37 °C.

Example 26. The porosity of a hardened sample of PCA calcium phosphate prepared according to Example 10-5 was determined.

A hardened sample of PCA calcium phosphate (1 g) was weighed immediately after removal from the moist incubator, and then air dried at room temperature for 12 hrs. The dried sample was carefully weighed and then the volume was calculated. The sample was placed into a 20 mL sample of water.

corresponding to Types 2 and 10 described in Example 11. The PCA calcium phosphate was pre-hardened in a moist environment at approx. 40 °C immediately prior to implantation. The control implants were 3 mm x 4 mm cylinders of silicone and porous hydroxyapatite, respectively.

Two adult female hound-type dogs (20 to 25 kg) were used in the study. Both dogs received two control implants (1 of each) on the right side of the mandible and one each of the Type 2 and Type 10 PCA calcium phosphate formulations on the left (opposite) side.

Implantation was performed under full anesthesia and aseptic surgical conditions. The animals were premedicated with tranquilizers and atropine-type agents and induced with barbiturates. The animal's vital signs (temperature, heart rate, respiratory rate) were monitored before and throughout the procedure. The animals were tested for proper anesthetic depth by toe pinch and corneal stimulus. After obtaining adequate anesthesia, using aseptic technique, an incision was made in the skin over the midlateral ventral surface of the mandible and proximal neck (over the mandible lower edge). The soft tissue was deflected away and the bone was exposed. The periosteum over the outer mandibular surface was elevated and the bone surface was roughened with a burr or drill until it was rough and bloody in a shape to accept the cylindrical implants. The control articles and pre-hardened PCA calcium phosphate were placed into the defects. Two samples per animal per side were onlaid onto each outer mandible surface using this method (two experimental PCA calcium phosphate samples and two controls). The samples were placed about 1 cm to insure that they do not appose each other. The periosteum was closed first using 3.0 vicryl. The soft tissues were then closed in layers with 3-0 vicryl absorbable suture. The skin was closed with simple interrupted sutures of 5-0 nylon. The animals were allowed to heal for scheduled periods of time. One dog was sacrificed at 3 weeks and the other at 3 months and the test sites were removed for histology. All animals were euthanized and identifying marks were collected.

The implantation sites were prepared as undecalcified sections. Sections were evaluated for biointegration, biodegradation, and biocompatibility.

The results were as follows: At all time points excellent biocompatibility

The four kinds of media chosen were: (-MEM (Minimum Essential Medium); TBS (Tris Bovine Serum: 50mM of Tris + 150mM of NaCl); (-MEM + FBS (Fetal Bovine Serum 10%); and Complete Media (immersion for 2h in TBS at 37C and subsequent immersion into the (-MEM + FBS).

A 0.3g sample of mixed precursors ACP and DCPD was compressed for one minute at 7 tons using the Carver Laboratory Press. The resulting pellet (a) had a diameter of 12mm and a height of 1mm. The pellet was put into 10ml of distilled water at 37°C for 30 minutes. After incubation, the pellet was put in the 6ml of different media at 37°C for 24 and 48 hours.

A second 1g sample of mixed precursors ACP and DCPD was combined with 0.8ml of distilled water. The mixture was rolled into a ball and dropped into 10ml distilled water at 37°C for 30 minutes. The ball was then ground using a mortar and pestle to obtain a fine powder. The powder was pressed for one minute at 7 tons using a Carver Laboratory Press. The resulting pellet (b) had a diameter of 12mm and a height of 1mm. The pellet was then put into the different media at 37C for 24 and 48 hours.

The pH of the solution of media was measured (at 25(C) at different times of 0, 24, and 48 hours after incubation at 37°C. The results of this study are displayed in Table 11.

Table 11. pH of Solution

Sample	α-MEM			TBS			α-MEM+FBS			Complete		
Preparation	0h	24h	48h	0h	24h	48h	0h	24h	48h	0h	24h	48h
a	7.6	8.1	7.9	7.5	7.0	6.8	7.5	7.7	8.2	7.6	7.9	7.9
b	7.3	7.3	7.1	7.3	6.5	6.0	7.4	7.5	7.5	7.5	7.5	7.3

Example 35. Reacting precursors, lyopholizing, crumbling, compressing. This example illustrates how a pellet is formed from PCA calcium phosphate paste.

PCA is made using ACP and DCPD, as the promoter. Saline is used as

the biologically suitable aqueous medium. The prepared PCA paste is then lyophol hardened in vitro at 37 °C and subsequently lyopholized. The hardened PCA material is then crumbled by hand. Once crumbled, the PCA material is formed into a pellet by methods described in Examples 32 and 33.

Example 36: Shaping, hardening, lyopholizing without grinding. This example shows how a pellet is formed from PCA calcium phosphate paste.

ACP and DCPD are selected as the precursors. An appropriate amount of Saline is used to make a PCA paste. The PCA paste is shaped into the desired form. It is then incubated at 37 °C in vitro for 30 minutes. The hardened object is then lyopholized.

Example 37. In vivo experiments comparing the methods. This example compares the methods of producing the pellets through *in vivo* experimentation.

Pellets are made according to Example 32. Two pellets are implanted into a dog femur. The animals are sacrificed and the implantation sites are analyzed for remaining residual material at time points of 3, 4 and 6 weeks. At each time point, decalcified and undecalcified slides of the implantation site are prepared and stained. These slides are histomorphometrically analyzed to determine the similarity of the prepared pellets to that of PCA calcium phosphate paste.

Example 38. Incorporation of a filler or binder. This example demonstrates the use of a filler to study plastic flow, with particular interest in the effect of tensile strength in the pellet.

A compressible sugar is used as a filler in conjunction with pellet production. The sugar is mixed with the precursors ACP and DCPD in a ratio of 1:1:1 before compression. The pellet is produced according to Example 33 with modifications in the duration of the total compression cycle and the duration of the maximum compressive force. The effectiveness of the sugar filler is measured by comparing the tensile strength of the pellets. The equation used to compute tensile strength is:

$$\sigma_0 = 2F/\Pi dt,$$

where σ_0 is the tensile strength, F is the force needed to cleave the tablet, d is the diameter of the pellet, and t is the tablet thickness or height.

Example 39: Delivery of a vaccine in a pellet. This example explains how the pellet is used as a delivery vehicle for a vaccine.

Keyhole limpet hemocyanin is prepared at a concentration of 0.5 mg/ml in phosphate buffered saline pH 7.0. 0.8 ml of this solution is added to 1 g of a 1:1 mixture of activated ACP and DCPD ... and mixed into a putty. The prepared PCA putty is then lyopholized. The dry material is milled for 10 minutes into a powder using a SPEX 8510 laboratory mill with a SPEX 8505 alumina ceramic grinding chamber. The powdered PCA is then prepared in a pellet as described in Example 32. A pellet formed by Example 32 is implanted subcutaneously in a rat. The process is repeated on a monthly basis for four months. Blood samples are taken on a regular basis and anti-Keyhole limpet hemocyanin antibody titers are determined by ELISA.

Example 40. This example describes the preparation of PCA calcium phosphate using alternative second calcium phosphate sources. Both pre-hardened PCA calcium phosphate and crystalline hydroxyapatite reacted with reactive amorphous ACP to produce a PCA calcium phosphate.

(a) Poorly crystalline HA is prepared as described in U.S.S.N. 08/554,817 filed November 7, 1995, incorporated herein by reference, using only carbonate as an inhibitor (no Mg^{++} or pyrophosphate). The resultant powder was then lyophilized.

(b) Hydroxyapatite was obtained in powder form from Aldrich Chemicals (#28,939-6; lot 00325AQ).

Each of the two powders was mixed 1:1 with reactive amorphous calcium phosphate, prepared as described in Example 5 and mixed with water. Both mixtures hardened within 30 minutes at 37 °C and IR spectra of the reaction products were substantially the same as that of the PCA calcium phosphate produced according to Example 8.

Example 41. This example describes the preparation of particulate PCA calcium phosphate which may be used in the composites of the invention.

Reactive amorphous calcium phosphate and DCPD are prepared as described in Examples 5 and 8 and are used to prepare poorly crystalline

hydroxyapatite as described in Example 8. The hardened PCA calcium phosphate is lyophilized overnight and pulverized in a grinder and then passed through one or more sieves to obtain a desired particle size. Particles are then introduced into a PLGA. A variety of composite matrices are prepared as follows:

(a) 25μm average particle size PCA calcium phosphate (10% wt/wt) in PLGA;

(b) 25μm average particle size PCA calcium phosphate (5% wt/wt) in PLGA;

(c) 100μm average particle size PCA calcium phosphate (5% wt/wt) in PLGA; and

(d) 200μm average particle size PCA calcium phosphate (5% wt/wt) in PLGA.

The composites prepared as above are placed intramuscularly in a rodent and resorption rates determined according to Example 16 to identify composites suitable for use in resorbable bioceramic composites.

Example 42. This example describes the preparation and testing of

resorbable PCA calcium phosphate composites.

A PCA calcium phosphate/poly(lactide) composite paste is prepared as described in Example 13 or Example 41. The paste is packed into molds in the shape of intermedulary nails, support plates, and screws. The molds are heated to 37 °C for three hours in a moist environment and the hardened objects are removed from the mold. The composite objects are implanted into animal models according to the procedure set forth in Example 19, in all cases being sure to contact the object with bone forming cells. Composites which are found to be fully resorbed and ossified in less than 6 months are suitable for use as bioresorbable bioceramic composite implants.

Example 43. This example describes a resorbable composite for use as a bone filler or cement. A PCA calcium phosphate/dextran composite may be prepared by first preparing the paste as described in Example 8. The paste may be well mixed with 10% vol/vol polydisperse dextran, hardened in a moist environment and shown to have improved strength and compressibility. The hardened composite may be then introduced into a fracture site in an animal model according to Example 19. The time for resorption and reossification are determined. Screening according to Example 29 is used to determine the suitability of the composite as a resorbable bioceramic implant.

Example 44. This example describes the coating of PCA calcium phosphate particles with a biodegradable outer coating. Particles prepared in this way resorb and/or ossify with an initial delay period as compared to PCA calcium phosphate alone.

PCA calcium phosphate particles may be prepared as described in Example 8. The particles may be prepared in a series of homogeneous lots with average particle sizes in the range of 60-100 microns according to the method used in Example 11. These particles may be then uniformly dip coated with poly(lactide). The coated particles are placed intramuscularly in order to evaluate the resorption kinetics, which may be delayed as compared to uncoated particles.

Example 45. This example describes the use of a PCA calcium phosphate/hydroxyapatite composite to produce new bone. This form of bone is useful in augmentation therapy.

Crystalline hydroxyapatite may be prepared or obtained as 50-200 micron particles. These particles may be introduced into a PCA calcium phosphate paste at approximately 1 to 50 wt% and may be well mixed. The resultant composite paste may be formed into the desired shape, seeded with bone forming cells and implanted adjacent to cortical bone and fixed by suturing and soft tissue approximation. The composite may also be seated on a recipient bone which has been surgically fashioned according to the method of Example 19. After three months, the implant site may be examined as in Example 19 to establish that the new bone impregnated with particulate hydroxyapatite is formed in the shape of the formed implant.

Example 46. This example describes the formation of a PCA calcium phosphate composite with a lubricant.

A PCA calcium phosphate paste may be prepared according to Example 8. Silicone oil may be mixed with the paste at a concentration in the range of 0.1 to 30 wt%. Before the hardening reaction occurs, the paste may be injected through a 16-22 gauge needle and found to have significantly improved injectability as compared to an untreated paste.

Example 47. This example demonstrates the use of a PCA calcium phosphate composite to embed an object in the recipient's bone. In addition to placement of anchoring devices, similar approaches can be used to embed almost any desired agent into a recipient's bone, including but not limited to support rods and fibers, imaging agents and friction reducing substances such as teflon plates.

A dacron loop approximately 1 mm in diameter may be formed on a 2 cm dacron suture. A knot may be placed within the suture approximately 2 mm from the loop. The suture may be then trimmed at the knot, leaving a loop with a 2 mm knotted tail. A 1 mm diameter hole may be drilled approximately 3 mm into a recipients's bone. The knotted end of the suture may be placed within the hole and the hole may be then filled with PCA calcium phosphate paste. After six months, suture site is evaluated for resorption of the PCA material in order to evaluate the composite's suitability as a resorbable bioceramic composite.

The procedure may be repeated in a second subject with the following modification. Following placement of the knotted suture within the hole, a

prehardened PCA calcium phosphate plug may be wedged securely into the hole, thereby mechanically securing the suture in place. The hole may be then sealed with poorly crystalline hydroxyapatite paste. After six months, suture site is evaluated for resorption of the PCA material in order to evaluate the composite's suitability as a resorbable bioceramic composite.

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Example 48. Efficacy Study of PCA in the Canine Alveolar Augmentation/Tooth Socket Model. This example demonstrates the use of the inventive PCA to restore bone tissue in an extracted canine tooth pocket.

The animals are premedicated with tranquilizers and atropine-type agents and induced and maintained with barbiturates. The animal's vital signs (temperature, heart rate, respiratory rate) are monitored before and throughout the procedure. The animal is then tested for proper anesthetic depth by toe pinch and corneal stimulus.

After obtaining adequate anesthesia, the gingival soft tissue is gently deflected away form the periphery of each premolar. The premolars are drilled in half with a slow speed dental drill and saline irrigation from the oral surface of the tooth to the lower surface between the roots. Each tooth half is then firmly grasped with extraction forceps and gently but firmly rotated until the tooth attachments are broken. The halves of each tooth are then removed. Bleeding is stopped by pressure and time. All premolars are extracted as described. After tooth removal and before PCA calcium phosphate placement, the lingual to buccal alveolar thickness is measured and recorded in at least 3 locations; these measurements are repeated after PCA calcium phosphate placement and at the time of necropsy and are used as a measure of bone ingrowth.

PCA calcium phosphate is prepared as Type 10 as described in Example 11. The empty tooth sockets/alveoli are located along one side of the mandible in the spaces formerly occupied by the premolar teeth. All dogs are implanted with PCA calcium phosphate in one side of the mandible and the opposite side remain untreated as unfilled controls. The gingival soft tissues are then closed in layers with 3-0 suture. After the surgical procedure the animals are monitored until they are stable.

The animals are allowed to heal for scheduled periods of time. Two dogs are sacrificed at 3 weeks, and two dogs are sacrificed at 2 months.

All animals are euthanized with a commercially prepared product used for

euthanasia (such as sodium pentobarbital), and the mandibles and identifying marks are then collected and preserved in 10% neutral buffered formalin or another suitable fixative for decalcified and undecalcified bone sections. Mandibles are measured as described above and radiographed. The test sites thereafter are removed for histology.

The implantation sites are prepared as decalcified and undecalcified sections. Sections are evaluated for biointegration, biodegradation, and biocompatibility.

A similar procedure was performed on a single dog. The implant was shown to bioresorb and to exhibit osseointegration within four weeks. Figure 26 is a photograph of a histological slide of the tooth socket implant site four weeks after surgery demonstrating the extent of bone ingrowth into the socket. The large arrows indicate the boarder between the natural bone 1 and implant site 2. Note the extensive ingrowth of bone tissue at site 2. The gingival tissue is indicated at 3.

Example 49. Osteoporotic Spinal Chord. This example demonstrates the procedure used for the treatment of osteoporatic vertebra.

A spinal cord was obtained from the cadaver of an osteoporotic patient. Injectable PCA was prepared as Type 10 described in Example 11 using 1.5 mls of water per gram PCA as a hydrating agent. A 16 gauge bone biopsy needle (a Quantico bone needle is also useful for this purpose) was inserted into the trabecular vertebral bone (Figure 27). A second 16 gauge needle affixed to an empty 50 cc syringe was inserted into the opposite side of the same vertebrae. Needle location was confirmed by X-ray (Fig. 27). Following confirmation of needle location, a syringe containing freshly hydrated PCA was affixed to the bone biopsy needle. PCA calcium phosphate was slowly injected from syringe with the biopsy needle simultaneous with slowly withdrawing the needle and applying gentle suction through the 50 cc syringe. The injected PCA can be seen as an electron dense area within the vertebrae in the X-rays in Figure 28b, as compared to the osteoporotic vertebrae before implantation (Fig. 28a). These results confirm the injectability of the inventive PCA calcium phosphate paste into the spinal cord of an osteoporotic patient.

Example 50. Canine anterior lumbar interbody fusion. This example describes the use of PCA calcium phosphate in the fusion of canine spinal vertebrae.

Animals were anesthetized, positioned in the right lateral decubitus position, shaved from anterior to posterior midline, extending form mid thorax to the pelvis. Following sterile prep and drape, a standard left retroperitoneal approach to the anterior lumbar spine was performed, with exposure of the L3-L6 vertebrae. The segmental vessels overlying L4 and L5 were ligated and divided, allowing anterolateral exposure of the L3-4, L4-5 and L5-6 discs. Anterior discectomies were performed at each level with the endplate prepared parallel and to bleeding subchondral bone using a parallel-paired-bladed oscillating saw (Aesculap). Following discectomy, a cylindrical titanium cage containing either PCA calcium phosphate or autologous bone or an unfilled cage was inserted into each disc space. Autogenous iliac crest bone graft was harvested from the left anterior iliac crest through a separate incision just prior to its packing into the cage and insertion into the disc space. After all three cages were inserted, internal fixation was applied using 4.5 mm vertebral body screws and a 6 mm diameter longitudinal rod from L3 to L6. Closure of the abdominal wound and iliac crest graft site was then done in layers using absorbable sutures and skin staples.

Dogs are sacrificed at two and twelve weeks and the histology of undecalcified sections are examined for evidence of new bone growth and vertebral fusion. Upon visual inspection on explant, the spinal cords using the PCA calcium phosphate of the invention appeared fused.

Other Embodiments

It will be understood that the foregoing is merely descriptive of certain preferred embodiments of the invention and is not intended to be limiting thereof. The following claims cover all of the generic and specific features of the invention herein described in the text and accompanying drawings.